IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No.: 2663
Steven M. RUBEN	Art Unit: 1644
Appl. No.: 10/662,429	Examiner: HUYNH, PHUONG N.
Filed: September 16, 2003	Atty. Docket: 1488.1890003/EJH/SAC
For: **Apoptosis Inducing Molecule I**	

Declaration of Solange Gentz
Ruben Exhibit #68

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Paper No. _____

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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES
(Administrative Patent Judge Sally Gardner Lane)

STEVEN M. RUBEN

Junior Party,
(Application No. 08/816,981),

v.

STEVEN R. WILEY
and RAYMOND G. GOODWIN

Senior Party,
(Patent No. 5,763,223).

Patent Interference No. 105,077

DECLARATION OF SOLANGE GENTZ

Ruben EXHIBIT 2068
Ruben v. Wiley et al.
Interference No. 105,077
RX 2068

DECLARATION OF SOLANGE GENTZ

1. I was employed by Human Genome Sciences, Inc. (HGS) from November 1993 to June 1998 and then from June 2000 through May 2002. I have been asked by patent counsel for HGS to describe my role and the experiments I carried out at HGS during the period from February 1995 through March 1996 on members of the TNF family of ligands and receptors. During the period from February 1995 through March 1996, I was a Research Associate at HGS in the Department of Protein Development. At that time, I was known as Solange H. Lima. I worked under the direct supervision of Dr. Timothy Coleman.

2. During the time period from February 1995 through March 1996, I carried out a large number of experiments to engineer and express TNF ligand and TNF receptor family members in insect cells using the baculovirus expression system. During this time, I directly carried out the amplification of a transfected CHO dhfr$^-$ line producing TNF gamma, as detailed below. This amplification was for the purpose of increasing the expression level of the recombinant TNF gamma protein for functional studies by HGS scientists. During this time period, I also prepared baculovirus vector encoding a soluble TNF receptor family member (clone HTOAU65) for recombinant expression of the protein in insect cells, as detailed below. In addition, I prepared baculovirus encoding two forms of AIM-1 protein (clones HTPAN08S04-51 bp and HTPAN08S04-185 bp, referred to by others at HGS as "FAS-51" and "FAS-185", respectively) for recombinant expression of AIM-I protein in insect cells. I recorded these experiments in my notebooks on the dates on which I performed such experiments. From time to time, my supervisor Dr. Coleman would review my notebook and memorialize his review of the experiments recorded therein by signing and dating my notebook, as was the standard business

practice at HGS at that time. In two instances, Dr. Reiner Gentz reviewed my notebook and memorialized his review of the experiments recorded therein by signing and dating my notebook.

3. I recorded the experiments I carried out during the April 1995 through March 1996 time period in my Notebook Number 279. RE69 contains copies of relevant pages of Notebook Number 279. During this period, Dr. Coleman signed and dated my work in Notebook Number 279, according to the practice described above, as follows:

Page	Date
75	October 13, 1995
93	November 28, 1995
99	December 19, 1995
105	February 1, 1996
115	February 28, 1996

In two instances, Dr. Reiner Gentz reviewed my notebook and memorialized his review of the experiments recorded therein by signing and dating my notebook, as indicated below:

Page	Date
33	April 19, 1995
42	May 19, 1995

4. On February 17, 1995, I transfected Sf9 cells with two recombinant baculoviral constructs for expression of AIM-I, designated HTPAN08SO4-51bp and HTPAN08SO4-185 bp (RE69, page 2). The transfected cells were cultured until February 22, 1995, when I harvested the transfections. On February 22, 1995, I performed baculovirus plaque purification on the cells infected on February 17, 1995(RE69, pages 4-5), according to the general procedure that I used at that time to isolate recombinant baculovirus containing the constructs. This general procedure for plaque purification, which is recorded in my notebook number 126 (RE70, page 6), required a 4-6 day incubation. Thus, the plaque purification work continued

from February 22, 1996 until at the very least February 26, 1996. Accordingly, this work represented continuous activity from February 17, 1995 through February 22, 1995.

5. On March 1, 1995, the plaque-purified baculovirus harboring the HTPAN08SO4-51bp construct and plaque-purified baculovirus harboring the HTPAN08SO4-185bp construct were used to infect Sf9 cells (1 RE69, page 9) to obtain recombinant baculovirus. The Sf9 cells infected with baculoviruses harboring the HTPAN08SO4-51bp and HTPAN08SO4-185bp expression constructs (referred to hereinafter as HTPAN08SO4-51bp baculovirus and HTPAN08SO4-185bp baculovirus for brevity) on March 1, 1995 were cultured until March 6, 1995 to amplify the isolated recombinant baculovirus. On March 6, 1995, I harvested the cells and assessed successful infection by a colorimetric assay to which I refer in my notebook as the "blue assay". The blue assay on both sets of HTPAN08SO4-51bp baculovirus-infected cells resulted in weak/negative signals while the blue assay on both sets of HTPAN08SO4-185bp baculovirus-infected cells resulted in positive signals, with the positive signals indicating the presence of recombinant virus (RE69 , page 12). Thus, this work represents continuous activity on this project from at least March 1, 1995 through March 6, 1995.

6. Due to the weak/negative signals obtained with HTPAN08SO4-51bp baculovirus in the March 1, 1995 infection, I re-infected Sf9 cells with this virus on March 8, 1995. The virus I used for this infection was the preparation of February 22, 1995 (RE69, page 15).

7. I harvested these cells on March 13, 1995 (1 RE69, page 17). Thus, this work represents continuous activity from March 8 through March 13.

8. I must have also infected Sf9 cells with HTPAN08SO4-185bp baculovirus on March 9, 1995, because on March 14, 1994, I harvested HTPAN08SO4-185bp baculovirus from

Sf9 cells infected on that date (RE69 , page 18). Thus, this work represents continuous activity on this project from at least March 9, 1995 through March 14, 1995.

9. On March 23, 1995, I infected a spinner flask (a large-scale culture) of Sf9 cells with the HTPAN08SO4-185bp baculovirus (RE69, page 22), and allowed the cells to grow until March 28, 1995, when I harvested the infected cells. I gave the cells and supernatant to "Rajeev" (*i.e.*, Rajeev Chillakuru of HGS's protein purification/analysis group) (RE69 page 24). Thus, this work represents continuous activity on this project from at least March 23, 1995 through March 28, 1995.

10. On March 30, 1995, I infected two more spinner flasks of Sf9 cells with the HTPAN08SO4-185bp baculoviral construct (RE69 , page 26), and allowed the cells to grow until April 4, 1995, when I harvested the cells, which I gave to Rajeev (RE69, page 28). Thus, this work represents continuous activity on this project from at least March 30, 1995 through April 4, 1995.

11. On April 11, 1995, I attempted to plaque-purify baculovirus carrying the HTPAN08S04-51bp construct which encoded the AIM-1 (RE69 , page 31). Baculovirus plaque purification was carried out according to my standard procedure that I used during that time period, and the plaques were grown until April 18, 1995. On April 18, 1995, I noted that the overlaid dishes containing Sf9 cells infected with HTPAN08S 04 51bp baculovirus were contaminated (RE69, page 33).

12. April 21, 1995, I repeated the plaque purification of HTPAN08S04 51bp baculovirus (RE69 page 34) and grew the plaques until at least April 27, 1995.

13. On April 27, 1995, I infected Sf9 (insect) cells with plaque-purified HTPAN08S04 51bp baculovirus (RE69 page 36) to produce a viral stock.

14. On May 3,1995, I harvested the Sf9 cells that had been infected on April 27, 1995 with HTPAN08S04-51bp baculovirus. I also noted that both of the HTPAN08S04-51bp baculovirus preparations were positive in the blue assay (RE69 , page 38).

15. On May 5, 1995, I infected Sf9 cells with 0.05 mL/flask of isolate number 2 of the plaque-purified HTPAN08S04-51bp baculovirus for the preparation of a larger stock of HTPAN08S04 51bp baculovirus (RE69, page 39) and cultured the cells until May 9, 1995. On May 9, 1995, I harvested the Sf9 cells infected with HTPAN08S04 51bp baculovirus on May 5, 1995, and collected the baculovirus-containing supernatant as a viral stock (RE69, page 39).

16. Thus, the HTPAN08 expression process that began at least by February 17, 1995 was a nearly continuous treatment and culturing experiment that spanned the entire period from February 17, 1995 through at least May 9, 1995.

17. On October 3, 1995, I transfected CHO cells with TNF gamma DNA (clone HUVEO91) inserted into two different expression vectors: pN346 and pCHO-1 to generate stable cell lines for recombinant expression of TNF gamma (RE69, page 72). Generating such stable cell lines requires many rounds of selection for the construct amplification in the cells.

18. On October 4, 1995, I harvested the CHO cells transfected with TNF gamma on October 3, 1995, and seeded them in selective media and cultured them until October 18, 1995 (RE69 page 73).

19. On October 18, 1995, I picked clones of TNF gamma that had been under selection since the October 4, 1995 transfection and cultured them in individual wells of a multiwell plate until October 26, 1995 (RE69, page 77).

20. On October 26, 1995, I passaged the clones picked on October 18, 1995 into wells containing different concentrations of methotrexate ("MTX") to amplify the TNF gamma CHO clones that I had picked on October 18, 1995. I cultured the clones under MTX selection until November 6, 1995 to select cell lines that stably express TNF gamma (RE69 pages 79-80).

21. On November 6, 1995, I examined the cultured TNF gamma CHO clones, pooled some of the cultures, and recultured them in MTX to amplify the clones, culturing them until November 10, 1995 (RE69, pages 83-84).

22. On November 10, 1995, I gave the MTX treated cultures prepared on November 6, 1995 and supernatants from them to Dan Bednarik, a scientist at HGS, to test the TNF gamma produced by these cultures for antiviral activity (RE69, page 86).

23. On November 15, 1995, I observed and recorded the condition of the various MTX treated cultures and the results of the antiviral activity results, and either split or pooled the cultures accordingly into new concentrations of MTX and continued to culture the cells to further amplify the clones. They were cultured until November 21, 1995 (RE69, page 88).

24. On November 21, 1995, I observed and recorded the condition of the various MTX treated cultures from November 15, 1995. Once again, I either pooled or changed the MTX media accordingly, and continued to culture the cells until November 28, 1995 (RE69 pages 89-90).

25. On November 28, 1995, I observed and recorded the condition of the various MTX treated cultures from November 21, 1995. Once again, I either pooled or changed the MTX media accordingly, and continued to culture the cells until December 4, 1995 (RE69, pages 92-93).

26. On December 4, 1995, I froze some of the MTX amplified TNF gamma CHO cultures from November 28, 1995 (RE69, pages 94-95).

27. On December 5, 1995, I observed and recorded the condition of the remaining TNF gamma CHO cells from November 28, 1995 and split them accordingly into MEM2+ medium with MTX for further amplification (RE69, pages 95-96).

28. On December 8 and 12, 1995, I froze the remaining MTX amplified TNF gamma CHO cells (RE69, pages 97 and 98).

29. Thus, the TNF gamma amplification process was a continuous treatment and culturing experiment that spanned the entire period from the October 3, 1995 transfection to final December 12, 1995 freezing.

30. On January 22, 1996, I thawed the frozen amplified TNF gamma CHO cells and seeded them on MEM2+ medium and cultured them until January 24 and 25, 1996 (RE69, page 100).

31. On January 24 and 25, 1996, I split the seeded amplified TNF gamma CHO cells into fresh MTX containing medium for further amplification, culturing until January 30, 1996 (RE69, page 102).

32. On January 29, 1996, I split seeded amplified TNF gamma CHO cells from January 24, 1996 into fresh MTX containing medium for further amplification, culturing until February 5, 1996 (RE69, page 104).

33. On January 30, 1996, I split seeded amplified TNF gamma CHO cells from January 25, 1996 into fresh MTX containing medium for further amplification, culturing until February 5, 1996 (RE69, page 105).

34. On February 1, 1996, I split seeded amplified TNF gamma CHO cells from January 30, 1996 into fresh MTX containing medium for further amplification, culturing until February 5, 1996 (RE69 , page 105).

35. On February 5, 1996, I observed and recorded the condition of the seeded amplified TNF gamma CHO cells from January 29 and 30, 1996 and split them into fresh MTX containing medium accordingly for further amplification, until February 9, 1996 (RE69 , page 106).

36. On February 7, 1996, I transfected into Sf9 (insect) cells a construct of clone HTOAU65, a soluble TNFR receptor family member, in baculovirus expression vector pA2gp, which I received from Jian Ni, a scientist at HGS, to produce recombinant baculovirus for expression of the soluble TNF receptor protein (RE69 , page 107). These cells were cultured until February 14, 1996.

37. On February 9, 1996, I observed and recorded the condition of the amplified TNF gamma CHO cells from February 5, 1996 and split them into fresh MTX containing medium accordingly for further amplification, culturing until February 15, 1996 (RE69 page 108).

38. On February 14, 1996, I harvested the TNF receptor (HTOAU65) transfected Sf9 cells to isolate virus-containing supernatant to plaque-purify the virus from the supernatant RE69 page 109).

39. On February 15, 1996, I observed and recorded the condition of the amplified TNF gamma CHO cells from February 9, 1996 and split them into fresh MTX containing medium accordingly for further amplification, culturing until February 23, 1996 (RE69 , page 110).

40. On February 22, 1996, I changed the media of the amplified TNF gamma CHO cells from February 13, 1996 (RE69', page 112).

41. On February 23, 1996, I observed and recorded the condition of the amplified TNF gamma CHO cells from February 15, 1996 and split them into fresh MTX containing medium accordingly for further amplification, culturing until February 28, 1996(RE69, page 113).

42. On February 28, 1996, I observed and recorded the condition of the amplified TNF gamma CHO cells from February 23, 1996 and split them into fresh MTX containing medium accordingly for further amplification, culturing until March 6, 1996 (RE69, page 115).

43. On March 6, 1996, I observed and recorded the condition of the amplified TNF gamma CHO cells from February 28, 1996 and split them into fresh MTX containing medium accordingly for further amplification, culturing until March 13, 1996 (RE69 pages 118-119).

44. On March 6, 1996, I carried out plaque purification of the TNF receptor (HTOAU65) transfected Sf9 cells from February 14, 1996 to isolate baculovirus containing the TNF receptor construct (RE69', page 119).

45. On March 13, 1996, I observed and recorded the condition of the amplified TNF gamma CHO cells from March 6, 1996 and split them into fresh MTX containing medium accordingly for further amplification (RE69, page 122).

46. On March 13, 1996, I also infected Sf9 cells with plaque purified baculovirus containing TNF receptor (HTOAU65) DNA from March 6, 1996 to produce a stock of recombinant baculovirus (RE69', page 123).

47. Thus, the TNF gamma amplification process that resumed in January 1996 after the freezing of the cells in December 1995 was a continuous treatment and culturing experiment that spanned the entire period from the thawing of the cells on January 22, 1996, through the culturing of the cells on March 13, 1996.

48. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application captioned above or any patent issuing thereupon.

Date: 6/24/2004

Solange Gentz